Supplemental Experimental Procedures

Cell lines and *miR-17-92* constructs

Conditional lymphoma and leukemia cell lines were derived from *Eμ-tTA/tet-O-MYC* mice. MYC inactivation was achieved with doxycycline treatment (20ng/ml). The *miR-17-92* fragment containing the microRNA coding region and 150bp flanking sequences was PCR amplified with Phusion High-Fidelity DNA Polymerase and cloned into pMSCV-puromycin or pMSCV-neomycin retroviral vectors (BglII/XhoI). The primers were:

miR1792-F: CGTAGATCTACTTCTGGCTATTGGCTCCTC

miR1792-R: ATCTCGAGCCGTTTTACACACCAACGAA

Virus production and infection of lymphoma/leukemia cells was performed as previously reported (Wu et al., 2007).

microRNA Real-time PCR

Total microRNA was extracted with miRNeasy microRNA extraction kit (Qiagen). The microRNAs were first reverse-transcribed and then quantified with TaqMan microRNA assay kits (Applied Biosystems) following manufacturer's protocols. Each sample was run in triplicate. U6 snRNA was used to normalize the data.

Flow cytometry

Cells were resuspended in 1 ml PBS and fixed with 2ml ice-cold ethanol. Cells were treated with RNase and propidium iodide and analyzed on a FACScan flow cytometer (Becton Dickinson). For apoptosis staining, cells were stained with 7-AAD and Annexin-V. FACS data was analyzed with FlowJo software (Tree Star).

MYC inactivation and reactivation

Cell lines were infected with MSCV control, MSCV-*miR-17-92* or LMP *miR-30*-based shRNAs for target gene knockdown. Brief inactivation of MYC was obtained with doxycycline treatment (20ng/ml) for four days and then doxycycline was washed away by resuspending in fresh media four times. Cells were analyzed daily for cell cycle distribution after propidium iodide staining.

Microarray Analysis

Control and *miR-17-92*-expressing cell lines were used for the microarray analysis. MYC ON and MYC OFF samples were collected at 0 hour and 48 hours after MYC inactivation. Total RNA was isolated, reverse transcribed and run on Illumina WG-6 murine high-density expression arrays by the Stanford Functional Genomics Facility. The arrays were read using Illumina Bead Studio 3.4. The data were then loaded into Genespring GX 10 for basic analysis. The MYC regulated-genes are genes differentially expressed by MYC ON *versus* MYC OFF in control lymphoma. The *miR-17-92*-regulated genes are genes differentially expressed in control *versus* *miR-17-92*-expressing lymphoma under MYC OFF condition. Overlapping genes from the two comparisons were cross-compared and shown in the Venn diagram. Genes upregulated or downregulated by both MYC and *miR-17-92* were also analyzed with the Database for Annotation, Visualization and Integrated Discovery (DAVID) for enrichment of GO terms. The dowregulated genes were further filtered for genes with at least 2 *miR-17-92* binding sites common to three microRNA target prediction softwares (miRanda, miRWalk and Targetscan).

Tumor transplantation

The conditional MYC lymphoma cell line 4188 was transplanted into either SCID or FVB/N host mice and allowed to grow to 1.5cm diameter (about 2 weeks) before MYC inactivation with doxycycline administration in drinking water (200ug/ml). The SCID host mice were used for tracking tumor size during regression. Tumor diameters were measured with a caliper. Tumor volume (V) was calculated as: $V = ab^2 / 2$, where a indicates length (mm) and b indicates breadth (mm). FVB/N host mice were used for monitoring tumor reoccurrence after MYC inactivation. All animal experiments were

approved by Stanford's Administrative Panel on Laboratory Animal Care (APLAC) and in accordance with national guidelines.

Western Blot, Immunohistochemistry, and SA-β-gal staining

Western blotting and immunofluorescence were performed as described (Wu et al. 2007). The following antibodies were used for Western blotting and IHC: MYC (9E10 for Western blot and Epitomics 1472-1 for IHC), Tubulin (Sigma), Ki67 (BD Phamingen), Hbp1 (Proteintech), Sin3b (Santa Cruz), Btg1 (Abgent), and Suv420h1 (Aviva). Bim and phospho-Histone H3 antibodies were from Cell Signaling. Antibodies for different forms of methylated lysine 20 of histone H4 were from ActiveMotif. MYC OFF tumor samples for IHC were collected at 4 days of doxycycline treatment. Pictures were taken with 20-40x objectives on Nikon E800 and Nikon E1000M microscopes using SPOT Advanced software.

Staining was performed as previously described (Wu et al., 2007). Briefly, cryosections of OCT embedded tumors or cultured cells were fixed in 0.5% glutaraldehyde in PBS for 5 minutes, washed in PBS containing 1mM MgCl2 and stained overnight at 37°C with 0.1% X-gal in PBS (pH 6.0) containing 1mM MgCl2, 1mM K4Fe(CN)6, 1mM K3Fe(CN)6. After staining, sections or cells were washed with PBS containing 1mM MgCl2. Bright field images were taken at 20-60X and were quantified using MetaMorph image analysis software.

Chromatin immunoprecipitation

The control and miR-17-92-expressing cells were treated with 1% paraformadehyde for 5 minutes for crosslinking. Around 30 million cells were sheared with a Diagenode Bioruptor UCD-200 sonicator for 35-50 cycles at 30 seconds on 30 seconds off per cycle. The mouse Sin3b antibody was used to immunoprecipitate sheared chromatin with rabbit IgG as negative control. Quantitative PCR was done with primers amplifying promoter regions of *Aurkb, Bub1b, Cdc6,* and *Mybl2* genes. Primers sequences are:

Aurkb: CTGATCGGGTTCCGTTGG, GGGCAGAAGGAAGCGAGT

Bub1b: ACGTTCGACATCACAAGTGC, GACCAAACTCCCCATCAGC

Mybl2: GCGGGAGATAGGAAAGTGGT, CGTGTCTGCAGGTCTGGTC

Cdc6: AGACCTGGGGCTGTCCTATT, TCTCCCGCCACAAATTCTAC

Multiple knockdown with shRNA

The individual knockdown of *miR-17-92* target genes was accomplished using LMP miR-30-based shRNAs with puromycin selection marker (OpenBiosystems). After confirming the knockdown using the respective shRNAs in the LMP backbone, the shRNA cassettes were transferred out of the LMP vectors and inserted into the pMSCV-neomycin, pMSCV-hygromycin, pBabe-zeocin or pBabe-blasticidin to have different selection markers to allow for simultaneous knockdown of multiple genes in the same cell. About 2-8 shRNAs were tested for each target genes. The following shRNAs were confirmed to effectively knockdown target genes and were used for the final experiments (*Hbp1* #2: V2LMM_91102, *Hbp1* #6: V2LHS_19565, *Sin3b* #9: V3LMM_482239, *Btg1* #5: V3LHS_365951, *Btg1* #6: V2LMM_99484, *Bim* #1: V2LMM_220682, *Suv420h1* #1: V3LHS_349916, and *Suv420h1* #7: V3LMM_445999). To clone the LMP *miR-30* cassette into vectors with different drug selection markers to facilitate simultaneous knockdown of multiple genes, the following primers were used with the size of all PCR products at around 250bp. The pMSCV-neomycin is a homemade version by swapping the puromycin resistance gene in pMSCV-puromycin with the neomycin resistance gene. It has the same cloning sites and orientation as pMSCV-puromycin.

pMSCV-neomycin (XhoI/EcoRI) for *Sin3b* shRNA:

miR30_SalI-F, AAATTTGTCGACTAGGGATAACAGGGTAATTGTTTG

miR30_MfeI-R, ATATATCAATTGAAAAAAGTGATTTAATTTATACCATTTTAATTC.

pMSCV-hygromycin (BglII/XhoI) for *Btg1* shRNA:

miR30_BglII-F, AAATTTAGATCTTAGGGATAACAGGGTAATTGTTTG

miR30_SalI-R, ATATATGTCGACAAAAAAGTGATTTAATTTATACCATTTTAATTC.

pBabe-zeocin and pBabe-blasticidin (EcoRI/SalI) for *Bim* and *Suv420h1* shRNAs, respectively:

miR30-Mfe-F, AAATTTCAATTGTAGGGATAACAGGGTAATTGTTTG

miR30_SalI-R, ATATATGTCGACAAAAAAGTGATTTAATTTATACCATTTTAATTC

Construction of the 3'UTR luciferase reporters

The 3'UTR fragments containing *miR-17-92* sites were cloned from mouse thymus tissues using PCR. The *miR-17-92* sites were identified with TargetScan and mutated with overlap extension PCR protocols. All PCR runs were done with Phusion High-Fidelity DNA Polymerase. The renilla luciferase was cloned into pMSCV-hygromycin and retrovirally delivered into tumor cells to serve as internal control. The wild type and mutated 3'UTR fragments were cloned into pMSCV-puromycin. Primers and overlap extension PCR protocols for cloning the reporter fragments are available upon request. Samples were collected three times and dual luciferase assay was carried out in triplicates with a Dual-Luciferase Reporter Assay System (Promega). The firefly luciferase readings were normalized with renilla luciferase readings to derive the firefly/renilla ratios. Data of wild type 3'UTRs were normalized with mutant 3'UTRs that were set to 1.